(16) Diluent 16 (0.13*M* sterile pyrogen-free sodium carbonate solution). Dissolve 14.0 grams of anhydrous pyrogen-free sodium carbonate (prepared as described in paragraph (a)(5) of this section) in 1,000 milliliters pyrogen-free, distilled water. Sterilize in an autoclave at 121 °C for 20 minutes.

[39 FR 18944, May 30, 1974, as amended at 40 FR 51625, Nov. 6, 1975; 50 FR 48397, Nov. 25, 1985; 53 FR 13401, Apr. 25, 1988]

§ 436.32 Pyrogen test.

(a) *Method 1*—(1) *Test animal.* Use healthy, mature rabbits weighing not less than 1,800 grams each that have maintained their weight on an antibiotic-free diet for at least 1 week under the environmental conditions specified in this section. House the animals individually in an area of uniform temperature (±3° C.) and free from disturbances likely to excite them. Do not use animals for pyrogen tests more frequently than once every 48 hours or prior to 2 weeks following their having been given a test sample that was adjudged pyrogenic. Before using an animal that has not been used for a test during the previous 2 weeks, condition it 1 to 3 days prior to pyrogen testing by conducting a sham test as directed in paragraph (a)(2) of this section, omitting the injection.

(2) *Procedure.* Using equipment and diluents described in § 436.31, as necessary, perform the test in an area where the animals are housed or under similar environmental conditions. On the day of the test: Withhold all food from the animals being used until after completion of the test, except that access to water may be allowed; and determine the ''control temperature'' of each animal by inserting the temperature-measuring device into the rectum of the test animal to a depth of not less than 7.5 centimeters and allowing sufficient time to reach a maximum temperature, as previously determined, before taking the reading. In any one test use only those animals whose control temperatures do not deviate by more than 1° C. from each other and do not use any animal with a temperature exceeding 39.8° C. The control temperature recorded for each rabbit constitutes the temperature from which any subsequent rise following the injection of the material is calculated. If the product is packaged for dispensing and is in a combination package with a container of diluent, dilute the product as directed in the labeling. Warm the product to be tested to approximately 37° C. Dilute the sample with sterile, pyrogen-free saline (prepared as described in § 436.31(b)(2)) to the appropriate concentration specified in the individual section for each antibiotic to be tested. Inject a test dose of 1 milliliter of the diluted sample per kilogram of rabbit weight into an ear vein of each of three rabbits within 30 minutes subsequent to the control temperature reading. Record the temperature at 1, 2, and 3 hours subsequent to the injection.

(3) *Evaluation.* If no rabbit shows an individual rise in temperature of 0.6° C. or more above its respective control temperature, and if the sum of the three temperature rises does not exceed 1.4° C., the sample meets the requirements for the absence of pyrogens. If one or two rabbits show a temperature rise of 0.6° C. or more, or if the sum of the temperature rises exceeds 1.4° C., repeat the test using five other rabbits. If not more than three of the eight rabbits show individual rises in temperature of 0.6° C. or more, and if the sum of the eight temperature rises does not exceed 3.7° C., the sample meets the requirements for the absence of pyrogens.

(b) *Method 2.* Proceed as directed in paragraph (a) of this section, except dilute the sample with pyrogen-free water (diluent 1).

(c) *Method 3.* Proceed as directed in paragraph (a) of this section, except dilute the sample with pyrogen-free water (diluent 1) and inject a test dose of 2.0 milliliters of the diluted sample per kilogram of rabbit weight.

(d) *Method 4.* Proceed as directed in paragraph (a) of this section, except inject a test dose of 0.5 milliliter of the diluted sample per kilogram of rabbit weight.

(e) *Method 5.* Proceed as directed in paragraph (a) of this section, except dilute the sample with pyrogen-free water (diluent 1) and inject a test dose of 0.5 milliliter of the diluted sample per kilogram of rabbit weight.

(f) *Method 6.* Proceed as directed in paragraph (a) of this section, except dilute sample with 0.05*N* sodium hydroxide (diluent 9).

(g) *Method 7.* Proceed as directed in paragraph (a) of this section, except dilute sample with sodium carbonate solution (diluent 13).

(h) *Method 8.* Proceed as directed in paragraph (a) of this section, except inject a test dose of 2.0 milliliters of the diluted sample per kilogram of rabbit weight.

(i) *Method 9.* Proceed as directed in paragraph (a) of this section, except dilute sample with pyrogen-free sodium carbonate solution (diluent 15).

(j) *Method 10.* Proceed as directed in paragraph (a) of this section, except dilute the sample with sodium carbonate solution (diluent 16).

[39 FR 18944, May 30, 1974, as amended at 40 FR 51625, Nov. 6, 1975; 45 FR 22921, Apr. 4, 1980; 50 FR 48397, Nov. 25, 1985; 53 FR 13401, Apr. 25, 1988]

§ 436.35 Depressor substances test.

Proceed as directed in the USP XX depressor substances test. Prepare the sample test solution as follows: For each antibiotic listed in the table below, select the appropriate diluent and test dose (concentration and volume). If the product is packaged for dispensing and is in a combination package with a container of diluent, dilute the product as directed in the labeling.

Antibiotic	Diluent[1]	Concentration of test solution[2]	Volume of test solution to be injected[3]
Bleomycin sulfate	4	[4]0.5	1.0
Capreomycin sulfate	4	3.0	1.0
Chlortetracycline hydrochloride	3	5.0	.6
Clindamycin phosphate	4	5.0	1.0
Daunorubicin hydrochloride	4	1.5	1.0
Dihydrostreptomycin sulfate	4	3.0	1.0
Doxorubicin hydrochloride	4	1.5	1.0
Doxycycline hyclate	4	5.0	1.0
Lincomycin hydrochloride monohydrate	4	3.0	1.0
Minocycline hydrochloride	4	5.0	.6
Plicamycin	3	0.050	1.0
Mitomycin	4	0.050	1.0
Oxytetracycline[5]	3	5.0	.6
Oxytetracycline hydrochloride	4	5.0	.6
Rolitetracycline	4	5.0	.6
Rolitetracycline nitrate	4	5.0	.6
Sodium colistimethate	4	3.0	1.6
Spectinomycin hydrochloride	4	15.0	1.0
Streptomycin sulfate	4	3.0	1.0
Tetracycline hydrochloride	4	5.0	.6
Tetracycline phosphate[5]	3	5.0	.6
Vidarabine monohydrate[5]	4	1.0	1.0

[1] Diluent number as listed in sec. 436.31(b).
[2] Milligrams of activity per milliliter.
[3] Milliliters per kilogram of body weight.
[4] The concentration of the test solution is expressed in units per milliliter in lieu of milligrams of activity per milliliter.
[5] To prepare the test solution, proceed as directed in the individual section of the antibiotic drug regulation in this chapter for the antibiotic to be tested.

[46 FR 60568, Dec. 11, 1981, as amended at 46 FR 61071, Dec. 15, 1981; 49 FR 5096, Feb. 10, 1984]

Subpart D—Microbiological Assay Methods

§ 436.100 Laboratory equipment.

Equipment should be selected which is adequate for its intended use and should be thoroughly cleansed after each use to remove any antibiotic residues. The equipment should be kept covered when not in use. Clean glassware intended for holding and transferring the test organisms should be sterilized in a hot air oven at 200–220° C. for 2 hours. Volumetric flasks, pipettes, or accurately calibrated diluting devices should be used when diluting standard and sample solutions.

(a) *Microbiological agar diffusion assay*—(1) *Cylinders.* Use stainless steel cylinders with an outside diameter of 8 millimeters (±0.1 millimeter), an inside diameter of 6 millimeters (±0.1 millimeter), and a length of 10 millimeters (±0.1 millimeter).

(2) *Plates.* Plastic or glass Petri dishes may be used, having dimensions of 20 by 100 millimeters. Covers should be of suitable material.

(b) *Microbiological turbidimetric assay*—(1) *Tubes.* Tubes which give satisfactory results and have uniform length and diameter should be used. If reusable tubes are employed, care must be taken to remove not only all antibiotic residues from the previous test but also all traces of cleaning solution.

(2) *Colorimeter.* Use a suitable photoelectric colorimeter at a wavelength of 530 millimicrons. Set the instrument at zero absorbance with clear,